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AMENDMENTS

In the Specification:

Please amend the specification as follows. Clean copies of the amended paragraph follow and a marked up version showing the changes to the specification is attached at the end of this Amendment.

1. Please replace the paragraph starting on page 13, line 3 with the following:

For purposes of exemplifying the invention, a simplified plan view of the substrate of a detection apparatus in accordance with the invention is shown generally at 20 in FIG. 1. The substrate 21 is formed of a base plate of material, e.g., a polymeric plastic, preferably having a flat top surface area 22 which surrounds a detection region 23. As discussed further below, the detection region 23 is formed of a microstructure having depressions, which as shown in FIGS. 1 and 2, may comprise adjacent ridges 25 separating depressions, preferably grooves 26, with the grooves disposed parallel to one another across the detection region 23. The width and depth of the grooves 26, and the spacing between the grooves as defined by the tops of the ridges 25, are selected to cause liquid crystal material to adopt a uniform orientation that is impressed upon it by the grooves and ridges. The size of the grooves and the spacing of the grooves are also selected such that adherence of a pathogen particle or clumps of particles of appropriate size will disrupt the uniform orientation of the liquid crystal material, causing a visible change in the appearance of a liquid crystal to signal the detection of the virus to an observer. Various conventional liquid crystal materials may be utilized, including nematic and smectic liquid crystal. The liquid crystals may be thermotropic or lyotropic phases. In general, groove widths and depths which are suitable to be occupied by viruses will be in the range of 5 to 500 nanometers (nm) and suitable spacing of the grooves 26

by the ridges 25 may also be in the same range. Where the selected pathogen is a bacteria, the width and depths of the grooves will generally be in the range of 0.1 micrometer (μm) to 10 μm to allow the grooves to be occupied by the bacteria. The grooves may be of various geometries, e.g., square, rectangular, triangular, or semicircular, and typically will be formed somewhat rounded or wedge shaped at nano-dimensions. The width of the grooves is preferably selected to be about the size of an individual pathogen particle, so that the particle will fit at least partially into a groove to occupy the groove. The depressions 26 may be formed in geometries other than linear, parallel grooves, e.g., as ellipsoids, truncated grooves, mixtures of grooves of different width, depth and shape, selected to act on the liquid crystal to orient it in the desired uniform orientation.

2. Please replace the paragraph starting on page 14, line 3 with the following:

The apparatus of the present invention may utilize the grooved substrate by itself in the form 20 shown in FIG. 1. The apparatus of the invention may also be utilized with a cover panel to cover the liquid crystal material, as further illustrated in the cross-sectional view of FIG. 2. The substrate 21 is formed in the same manner as described above, having the grooves 26 spaced by ridges 25 of selected and controlled dimensions. A spacer or gasket 30 is mounted on the non-textured surface 22 to fully or partially surround the detection region 23 and to support a cover plate 31. The substrate 21, spacer 30 and cover plate 31 enclose a volume 32 which can contain the liquid crystal material.

3. Please replace the paragraph starting on page page 16, line 22 with the following:

For purposes of exemplifying the invention, a simplified plan view of the substrate of a detection apparatus in accordance with the invention is shown generally at 20 in FIG. 1. The substrate 21 is formed of a base plate of material, e.g., a polymeric plastic, preferably having a flat top surface area 22 which surrounds a detection region 23. As discussed further below, the detection region 23 is formed of a microstructure having depressions, which as shown in FIGS. 1 and 2, may comprise adjacent ridges 25 separating

depressions, preferably grooves 26, with the grooves disposed parallel to one another across the detection region 23. The width and depth of the grooves 26, and the spacing between the grooves as defined by the tops of the ridges 25, are selected to cause liquid crystal material to adopt a uniform orientation that is impressed upon it by the grooves and ridges. The size of the grooves and the spacing of the grooves are also selected such that adherence of a pathogen particle or clumps of particles of appropriate size will disrupt the uniform orientation of the liquid crystal material, causing a visible change in the appearance of a liquid crystal to signal the detection of the virus to an observer. Various conventional liquid crystal materials may be utilized, including nematic and smectic liquid crystal. The liquid crystals may be thermotropic or lyotropic phases. In general, groove widths and depths which are suitable to be occupied by viruses will be in the range of 5 to 500 nanometers (nm) and suitable spacing of the grooves 26 by the ridges 25 may also be in the same range. Where the selected pathogen is a bacteria, the width and depths of the grooves will generally be in the range of 0.1 micrometer (μm) to 10 μm to allow the grooves to be occupied by the bacteria. The grooves may be of various geometries, e.g., square, rectangular, triangular, or semicircular, and typically will be formed somewhat rounded or wedge shaped at nano-dimensions. The width of the grooves is preferably selected to be about the size of an individual pathogen particle, so that the particle will fit at least partially into a groove to occupy the groove. The depressions 26 may be formed in geometries other than linear, parallel grooves, e.g., as ellipsoids, truncated grooves, mixtures of grooves of different width, depth and shape, selected to act on the liquid crystal to orient it in the desired uniform orientation.

4. Please replace the paragraph starting on page 17, line 17 with the following:

An example of the invention will now be described with reference to FIGS. 3-8. This example utilized an elastomeric substrate 21 formed by nanoscale molding of polydimethylsiloxane, using as a mold the surface of a silicon wafer that was patterned with grooves having approximately 100 nm width and depth and 100 nm spacing that were formed by using e-beam lithography. The resulting microstructure with substantially square grooves and ridges of 100 nm dimensions is illustrated in the simplified cross-sectional view of FIG. 3.

Atomic force microscopy and scanning electron microscopy confirmed that the nanometer-scale topography of the silicon template was reproduced into the polymeric material. A layer 33 of BSA (as a blocking layer) and immunoglobulin G (IgG) (as a binding agent) was formed in a film on the surfaces of the grooves 26 and the ridges 25 in the detection region. One skilled in the art will recognize that portions or fragments of immunoglobulins may be used in place of the whole immunoglobulin. As shown in FIG. 3 for illustration, the size of the BSA and IgG molecules adhered to the surfaces to form the layer 33 was small in comparison to the dimensions of the grooves 26. The dimensions of the grooves 26 were comparable in size to vesicular stomatitis virus (VSV) (typically virus particle size about 100 nm x 45 nm), a particle of which is shown for illustration at 35 in FIG. 3. To form the layer 33, the patterned substrate 21 is sequentially immersed first in an aqueous solution of IgG and then in an aqueous solution of BSA. The resulting layer of molecules on the substrate surface is schematically illustrated in FIG. 5. When the substrate 21 with the layer 33 formed in this manner had a layer of liquid crystal material placed on it, the detection region in which the nanometer-scale grooves were formed caused the liquid crystal to appear uniformly dark when viewed between cross-polarizing sheets (with the grooves parallel to the analyzer). A commercially available liquid crystal material was utilized, 4-cyano-4'-pentylbiphenyl (5CB) nematic liquid crystal manufactured by BDH and available from EM Industries, Hawthorne, New York. (Other liquid crystal materials may be used, including smectic liquid crystals such as 8CB.) FIG. 4 shows the nano-textured detection region 23, the flat untextured area 24, and the region of the spacer 30. A photograph through the analyzer showed that the detection region 23 appeared uniformly dark due to uniform anchoring of the liquid crystal whereas the flat regions 22 of the substrate surface that do not have grooves were brightly colored because there are no grooves and the liquid crystal is not uniformly anchored on the surface. Thus, the presence of the nanoscale grooves 26 in the surface of the substrate in the detection region cause the liquid crystal to adopt a uniform orientation which is not erased by the adsorption of the BSA and IgG onto the substrate surface in the layer 33.

5. Please replace the paragraph starting on page 18, line 23 with the following:

FIGS. 5-7 schematically illustrate the results of an example utilizing such a substrate in combination with liquid crystals to detect the presence of a specific strain of virus, the Indiana strain of VSV. The surfaces of the substrate 21 with the grooves 26 formed therein was pretreated as described above to form a layer 33 of BSA and IgG, the IgG selected to specifically bind to the Indiana strain of VSV (i.e., anti-VSV-I IgG), as illustrated schematically in FIG. 5. The width of the grooves 26 in the substrate was 100 nm, which is on the order of the size of the VSV virus particle (about 100 nm x 45 nm), allowing the virus particle to at least partially fit into and occupy the groove. A photograph and observation of the detection region only which has the liquid crystal 5CB in contact therewith, showed a uniform dark appearance indicating the uniform anchoring of the liquid crystal to the substrate surface in the detection region. Another substrate similarly pretreated with BSA and anti-VSV-I IgG then was treated by placing a droplet of buffer containing the Indiana strain of VSV onto the surface of the substrate. The droplet was confined between a glass cover slip and the surface of the substrate. The cover slip was then removed, the surface of the substrate was rinsed with phosphate buffered saline (PBS), then placed under a stream of nitrogen to displace excess PBS, and then contacted with 5CB liquid crystal. When the droplet of buffer contained about 106 pfu/ml of VSV-I, the VSV-I was bound to the IgG, as illustrated schematically in FIG. 6, and the liquid crystal in contact with the surface then appeared non-uniform and bright. This result indicates that the presence of the virus erases the effect of the nanoscale grooves 26 on the alignment of the liquid crystal. A further experiment was performed utilizing the New Jersey strain of VSV, which does not bind to the IgG that is specific to the Indiana strain, as illustrated schematically in FIG. 7. When the substrate treated in this manner had liquid crystal applied to it, the uniform orientation of the liquid crystal was not substantially altered. The above results demonstrate that the detection system may be used to identify the presence of the Indiana strain of VSV in a sample and that the detection apparatus can differentiate between different strains of the same virus. Thus, the assay is specific to the Indiana strain of VSV.